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# THE HERMAPHRODITE GLAND AND GERM CELLS OF *VALLONIA PULCHELLA* MÜLL.*

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## INTRODUCTION

THE numerous studies of hermaphrodite mollusks have brought to light a variety of interesting and unusual reproductive conditions. Of particular interest relative to this paper is the capacity for self-fertilization revealed by some of the forms studied. Though in general hermaphroditism in this group has been regarded as of the "insufficient" type, the laying of fertile eggs in strict isolation has been recorded in a number of instances. Ikeda (1937) lists reports of this kind covering thirty species of Pulmonata, belonging to ten different genera. Some authors have questioned how far these laboratory results could be considered indicative of natural processes. For the most part they have been obtained with animals which copulate freely in nature.

The study presented here concerns *Vallonia pulchella* Müll., a small land snail, which gives evidence of a natural physiological isolation in contrast to one artificially brought about. Steenburg (1918) and Watson (1919) describe the hermaphrodite organs from minute dissections and call particular attention to the absence of male ducts in the majority of the specimens examined. A similar reduction of male organs has been found in *Acanthinula aculeata* (Boycott, 1917; Steenburg, 1918) and also in *Bullinus contortus* (Larambergue, 1930, 1932, 1933). In some cases the aphallic state is interpreted by Boycott as being a structural simplification associated with diminution in absolute size.

Elimination not only of male ducts but of male cells, resulting in parthenogenesis, has been reported for *Paludestrina jenkinsi* by Robson (1923) and for *Campeloma rufum* by Van Cleave and Altringer (1937) and Mattox (1937).

In a preliminary paper I have described the egg-laying habits of *Vallonia pulchella*, the hatching of the eggs, and the growth of the young to maturity (Whitney, 1938). Under laboratory conditions isolated individuals laid an average of one egg a day over a period of several months. The young were well developed and active when hatched. Desiccation and low temperatures were found to have an important modifying effect upon the egg-laying function and upon development. Copulation was never observed. Individuals isolated from the time of hatching laid eggs from which young hatched, and some of the latter reared in isolation reproduced

in like manner. This observation continued for several successive generations.

These macroscopic observations formed a basis for the present microscopic study to determine further details of reproduction in *Vallonia pulchella*, particularly those relating to self-fertilization. The possibility of parthenogenesis was not entirely excluded, even though male cells were present, since in many hermaphrodites various barriers against self-fertilization exist.

Some of the interesting phases of the problem concerned the origin and differentiation of male, female, and nutritive cells; possible abnormalities of maturation resulting in atypical sperm and ova, such as have been reported for other mollusks; possible cyclic conditions; modifications imposed by the small size of the animal, and by environmental factors; and, finally, the survival value of the conditions found, so far as this could be inferred. Self-fertilization and parthenogenesis have been cited as contributing factors in bringing about wider distribution of certain molluscan forms, especially under the influence of civilization (Robson, 1923; Boettger, 1931). Observations of *Vallonia* suggested similar interpretations.

I take this opportunity to express appreciation to Professor Peter Okkelberg for having directed this study, to members of the Departments of Zoölogy and Botany and of the Museum of the University of Michigan for valued suggestions and assistance, and to members of the Botany and Zoölogy Departments of Butler University, Indianapolis, Indiana, for many favors.

## GENERAL REVIEW OF THE LITERATURE

The problems studied necessitate a review of some of the literature bearing on hermaphroditism and self-fertilization among the mollusks in general and the pulmonates in particular. References having to do with more detailed aspects of the study will be reserved for sections in which they apply.

Furrow (1937), reviewing the work on sex in the Mollusca, distinguishes two types of hermaphrodite glands: the "segregated," in which there is an anatomical separation of male and female elements; and the "unsegregated," in which male and female cells arise within the same cysts. He points out, however, that in the latter there is a segregation in the time of development of male

and female cells which prevents self-fertilization. The intervals vary considerably, and under experimental conditions may be shortened almost to the point of complete elimination, when self-fertilization becomes possible. Furrow cites examples of such experimental alterations of the sexual cycle in the pond snails *Planorbis* and *Lymnaea*. Crabb (1927, 1928), however, expresses the view that self-fertilization is the normal, not the unusual, method of reproduction in the species of *Lymnaea* which he studied.

There has been some disagreement regarding barriers against self-fertilization. If such barriers are postulated, production of fertile eggs by isolated hermaphrodites must be attributed to parthenogenesis. As evidence in favor of self-fertilization rather than parthenogenesis in some of the forms studied authors have cited the presence of ripe sperm and eggs in the same cysts at practically all times, the giving off of two polar bodies, the development of two pronuclei, and the numerical ratios exhibited by the offspring of isolated heterozygotes (Crabb, 1927; Larambergue, 1930; Ikeda, 1937). In the parthenogenetic snail *Campeloma rufum*, in which no male elements whatever were to be found, Mattox (1937) describes a type of oögenesis in which only one polar body is produced, with no evidence of synapsis or reduction.

In general, one gathers from a survey of the literature that conditions in many hermaphrodite mollusks are such as to permit self-fertilization in varying degrees, and that in some this phenomenon may possibly occur naturally in combination with cross-fertilization.

## MATERIALS AND METHODS

For this study specimens of *Vallonia pulchella* Müll. were collected from typical locations in Ann Arbor, Michigan, and Indianapolis, Indiana. Some were reared in the laboratory. Microscopic preparations were made of adult specimens taken throughout the year, with a view to correlating histological conditions of the ovotestis with the seasonal activity observed. In order to trace the development of the gland juvenile specimens of different sizes were prepared. This series was graded from 0.6 mm., the size of individuals measured shortly after hatching, to 2.0 mm., the average adult size — the measurements representing the widest shell diameter of alcohol specimens.

The material was fixed in modified Bouin's solution, which de-

calcified the shell, so that it was easily removed. Normal butyl alcohol was used in the dehydration and infiltration of some of the specimens and dioxane for the rest. Serial sagittal sections gave the most favorable disposition of the organs for study. The material was sectioned for the most part at 8 or 10 microns, and stained with Heidenhain's iron hematoxylin without counterstain.

The small size was of some advantage for microscopic study in that a complete individual series could be accommodated on one or two slides, and the entire hermaphrodite gland could be studied *in situ*. In larger forms it must be removed and divided.

The figures were prepared from photomicrographs and from camera lucida drawings.

## ANATOMY OF THE HERMAPHRODITE ORGANS

The hermaphrodite organs of *Vallonia*, as described by Steenburg (1918), include the hermaphrodite gland, the hermaphrodite canal, the seminal canal, which is continuous with the vas deferens and penis, the narrow oviduct and vagina, and the genital cloaca, which opens by way of the common genital orifice behind the right tentacle. The prostate and the albumen gland and other accessory structures are also described. We have mentioned above that both Steenburg (1918) and Watson (1919) found the penis and vas deferens lacking in many specimens. This observation was confirmed by the present study, since careful examination of sections failed to reveal anything resembling these structures.

The hermaphrodite gland, or ovotestis, is embedded in the lower portion of the upper division of the liver. Steenburg (1918) describes its form as follows: "The pyriform acini are united in three groups, not very distinct, comprising three or four acini each." A gonad of this description is of the "unsegregated" type as distinguished by Furrow (1937). The cysts containing both male and female germ cells join the hermaphrodite duct, which serves as both a sperm duct and an oviduct. This condition is found in *Helix, Physa, Lymnaea, Planorbis,* and *Polygyra,* as well as in *Vallonia.*

## HISTOLOGY OF THE ADULT GLAND

Sagittal sections of mature animals collected during the active season show quite clearly the three groups of acini making up the ovotestis, as described by Steenburg (Pl. I, Fig. 1). The cortical

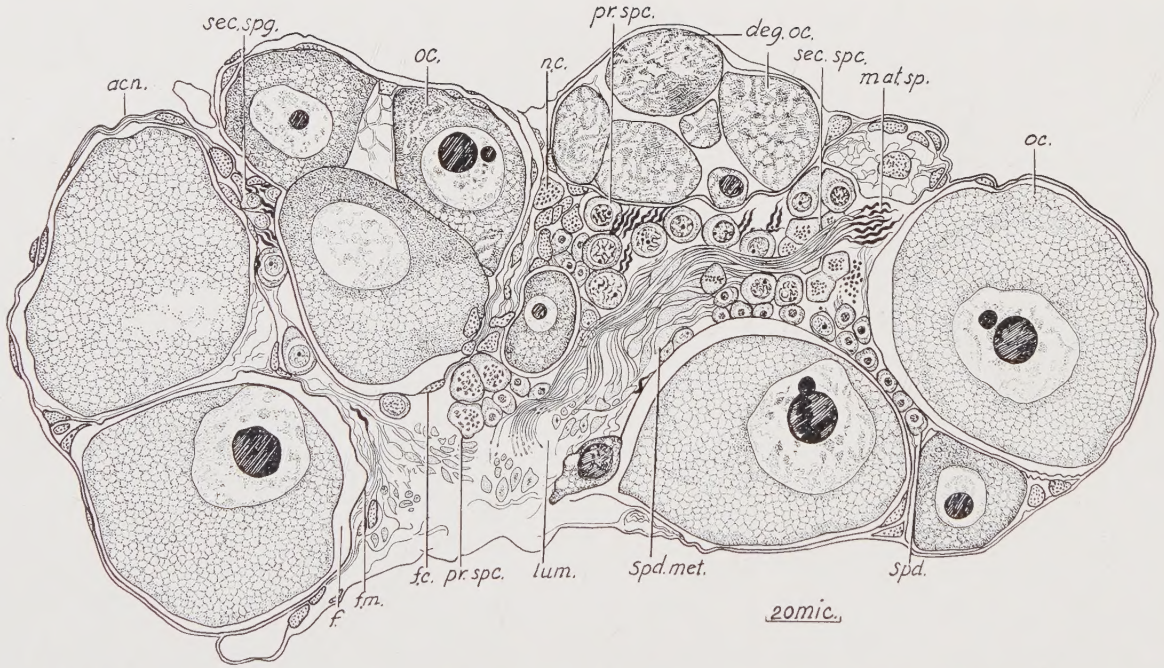


FIG. 1. Camera lucida study of mature gonad in individual collected in July. Abbreviations: *acn.*, acinus wall; *deg. oc.*, degenerating oöcytes; *f.*, follicle; *f. c.*, follicle cell; *f. m.*, follicular membrane; *lum.*, lumen; *mat. sp.*, mature spermatozoa; *n. c.*, nutritive cell; *oc.*, oöcytes; *pr. spc.*, primary spermatocytes; *sec. spc.*, secondary spermatocytes; *sec. spg.*, secondary spermatogonia; *spd.*, spermatids; *spd. met.*, spermatids in metamorphosis. (Reconstructed from three adjacent sections)

regions are crowded with large oöcytes protruding from the walls of the acini (text Fig. 1). These are covered with thin follicular membranes, in which at intervals isolated nuclei of various sizes appear. Some of the smaller nuclei are much flattened. The larger ones, of the nurse-cell type, are more rounded, and their chromatin has the characteristic appearance of coarse angular blocks. Cell outlines are difficult to distinguish. According to Gatenby (1919a) and Woldt (1932), the large "hyperchromatic" nurse cells apparently develop from smaller epithelial cells of more generalized type. Gatenby found all gradations between them.

It has been suggested that these nutritive or follicle cells, by building the follicular membranes, prevent self-fertilization; and again that they serve as supporting cells, holding the ova in position so that they remain advantageously situated with respect to the nutritive fluids of the body cavity, which must diffuse from the outside to the interior of the gland.

In *Vallonia*, as in *Helix* and other forms, it seems probable that additional nutritive material is contributed by degenerating oöcytes, which are very commonly found in the ovotestis. Some sections may show the whole contents of a cyst made up of these (text Fig. 1), or a cyst may contain one large developing oöcyte and several smaller degenerating ones.

Additional nutritive material may be contributed by spindle-shaped fragments of cytoplasm often observed in the neighborhood of mature spermatozoa and spermatids in various stages of metamorphosis (text Fig. 4p). It seems likely that this material, discarded during the process of metamorphosis, helps to form a nutritive fluid which fills the interior of the gland and bathes the cells.

The lumen of the active gland is occupied by male cells in various stages of maturation, especially in the medullary portion, where the acini converge toward the hermaphrodite duct. This seems to be the most active region in the production of new elements, for here small new oöcytes also appear in the germ wall, in proximity to the male cells in the lumen. From this position the small oöcytes apparently migrate, as they grow, into the deeper portions of the acini. Many of them exhibit an elongated, tapering form which suggests an ameboid migration, somewhat similar to that described for the germ cells of *Planorbis* by Merton (1930).

The male cells tend to occur in groups, although some isolated ones are scattered about, here and there. The cells composing each group are all in approximately the same stage of development, a condition noted in *Helix* by Demoll (1912) and others. Such groups have been called "cell nests," and each one has arisen, according to interpretation, from a single primary spermatogonium.

Fifteen to twenty large oöcytes, besides a number of smaller ones, have been counted in typical specimens collected in July. These quite fill up the acini and, together with the groups of male cells crowding the lumen, give the whole gland a relatively compact appearance.

Sections of material fixed during the dormant season, in January, for example, show the gland in a somewhat ragged form, with open spaces left by the discharge of cells during the previous season (Pl. II, Fig. 2). A number of large oöcytes may be left over, and some of medium size, but few if any small new ones are to be found. Male cells are few and scattered, not exhibiting the orderly arrangement in groups which is indicative of recent division. Throughout the gonad many of the cells appear shrunken and distorted, but others do not differ markedly from corresponding stages found during the active period. Conceivably they are in an arrested condition and might regain activity and function the following season.

Although egg laying did not take place in the out-of-door sites to any great extent until the middle of May, individuals collected early in March and April show evidence of considerable growth activity in the hermaphrodite gland, doubtless in response to warmer temperatures during a part of the day. In particular, the active medullary region is occupied by a considerable number of small oöcytes at the onset of their growth, and by early spermatocytes as well (Pl. II, Fig. 3).

Material fixed in the fall shows fewer cells and more open space (Pl. II, Fig. 4) than the summer material. Later growth and maturation stages are more conspicuous than earlier ones.

Animals reared or kept for some time in the laboratory under what might be regarded as optimum conditions are characterized in section by the same abundance and variety of growth and maturation stages as those collected during the active season out of doors.

THE DEVELOPING GLAND

The classic work of Ancel (1902) on *Helix pomatia* describes the genital rudiment as it appears within the mesoderm several days before hatching. At this early stage it is a quite solid mass of cells, which slowly elongates and ultimately fuses with the hermaphrodite canal. A lumen then appears which transforms the gonad into a hollow vesicle, united at one end with the hermaphrodite duct and expanded at the other. In Ancel's opinion, all the cells making up the genital gland are derived from elements of the primordial anlage. At no time do foreign elements penetrate into it. According to his observation, sperm cells were the first to differentiate, then nurse cells, and then egg cells, but Buresch (1911) describes these three elements as arising simultaneously from the germinal epithelium.

Pennypacker (1930) found both male and female germ cells in the ovotestis of *Polygyra appressa* from the 5-mm. stage on. These authors note that mature spermatozoa are present in the young gland of both *Helix* and *Polygyra* long before mature ova, and that the development of the male cells takes place in the lumen, whereas the female cells remain in the walls of the acini during their entire stay in the ovotestis.

The early development of the hermaphrodite gland of *Vallonia pulchella* presents striking similarities to that described by Ancel for *Helix*. In the present study the genital rudiment was first identified about the time of hatching. The stages representing the course of development are described and figured (Pl. III, Figs. 1–7). The time required to complete development varies somewhat with external conditions, so that size was regarded as a better descriptive criterion of the series than age.

*The 0.6-mm. Stage* (Pl. III, Fig. 1; Pl. IV, Fig. 1; text Fig. 2)

The newly hatched animal measures 0.5 to 0.6 mm. in diameter. It emerges well developed and active, with a whorl of its body shell completed. At this time the hermaphrodite gland is a small compact mass of dense, closely clustered cells, situated in the inner coil of the body. The cells are very small, scarcely more than 5 microns in diameter, and have proportionately large nuclei and very little cytoplasm. They are essentially alike in appearance, although varying somewhat in size. It is not possible at this stage to recognize

FIG. 2. Camera lucida study of immature undifferentiated gonad at time of hatching. Abbreviations: *h. d.*, hermaphrodite duct; *prim. g. c.*, primordial germ cells; *sh.*, sheath of gonad. (Reconstructed from two adjacent sections)

which cells will mature into male and which into female germ cells. In some preparations they appear grouped in a few small grapelike clusters, as if they had arisen by division from a still smaller number of cells of earlier origin. The whole group is invested with a sheath containing small darkly staining nuclei. Ventrally the genital rudiment tapers off into the very slender, slightly wavy hermaphrodite duct, which at this time also appears to be a solid rudiment. This stage reminds one of the first small mass of cells described by Ancel (1902) for *Helix*. Hsiao (1939) describes a similar undifferentiated early gonad for the marine snail *Limacina retroversa*.

*The 0.9-mm. Stage* (Pl. III, Fig. 2; Pl. IV, Fig. 2)

In animals measuring 0.9 mm. the massive arrangement of the gland persists, but cell differentiation is now initiated. At the periphery of the mass a few cells show the beginning of growth and are clearly recognizable as oöcytes because of the proportion of

cytoplasm to nucleus and the prominent double nucleoli, which are characteristic of oöcytes both large and small wherever found in the gland. At the center of the group are smaller cells, many with interphase nuclei, but a number in prophase and some with distinct chromosomes. These are the primary spermatogonia, and sometimes there are associated with them still smaller cells of similar appearance, the secondary spermatogonia. Thus the characteristic orientation of female cells toward the periphery and male cells in the interior becomes evident in the very young gland, in which no definite lumen is as yet formed. A few nurse-cell nuclei are irregularly disposed toward the outer portion of the cell group.

*The 1.0-mm. Stage* (Pl. III, Fig. 3; Pl. IV, Fig. 3; text Fig. 3)

In most preparations of 1.0-mm. animals or those a little larger there is a definite lumen in the hermaphrodite gland, so that it has the form of a simple undivided vesicle, which tapers below into the hermaphrodite duct. One is impressed with its similarity to the early gonad of *Helix* as described by Ancel. The older oöcytes are larger than those of previous stages, and small new ones occur in the portion of the gland nearest the hermaphrodite duct. As they grow they apparently migrate toward the deeper expanded portion of the germinal vesicle, in which region the largest ones are found. In the lumen are male cells in various stages. A number of ripe spermatozoa are included. Their early appearance in *Helix* and *Polygyra* has been noted.

There is evidence of an occasional penetration of these early maturing sperm cells into young oöcytes. At least some sections show bodies in the egg cytoplasm which are in every way similar to surrounding sperm heads, and at the periphery of these oöcytes are sperm heads which give the appearance of having just penetrated the surface by means of spiral movements (Pl. IV, Fig. 3; text Fig. 3). It is difficult to say what such an early impregnation may signify. In the slug *Arion empiricorum*, Lams (1910) has noted that oöcytes are regularly inseminated while still in the germ wall, and maturation and cleavage stages occur in the interior of the gland. Buchner (1914) describes a penetration of spermatozoa into the early oöcytes of the archiannelid *Saccocirrus*, the sperm being retained in the egg cytoplasm throughout the growth period and then taking part in an entirely normal fertilization.

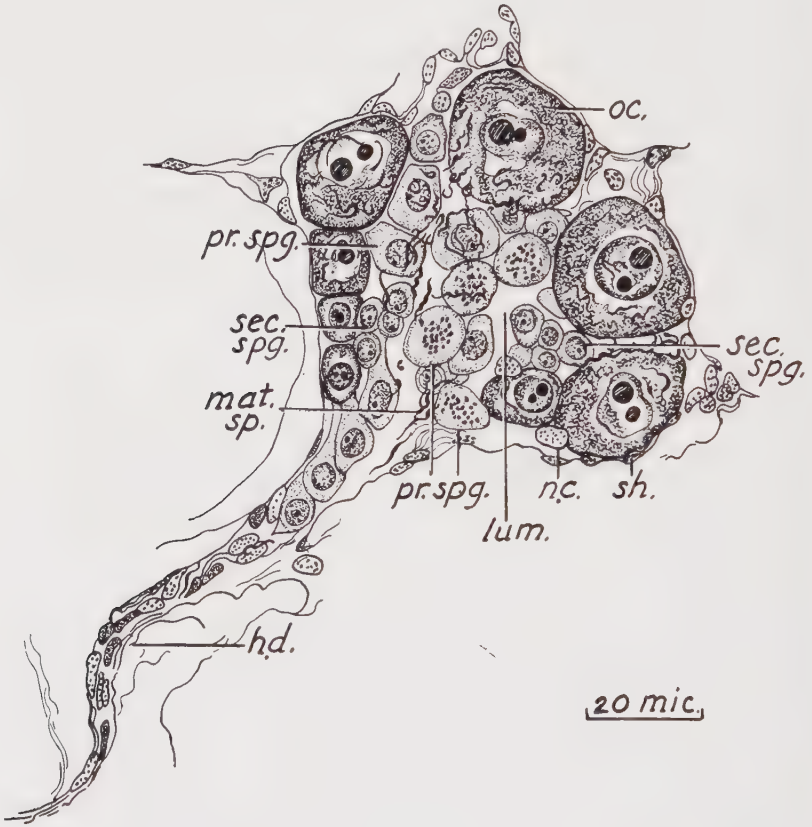


FIG. 3. Camera lucida study of immature gonad from 1.0-mm. individual, showing presence of mature spermatozoa and their penetration into young oöcytes. Abbreviations: *h. d.*, hermaphrodite duct; *lum.*, lumen of the gland; *mat. sp.*, mature spermatozoa; *n. c.*, nutritive cell; *oc.*, oöcyte; *pr. spg.*, primary spermatogonia, interphase and metaphase stages; *sec. spg.*, secondary spermatogonia; *sh.*, sheath. (Reconstructed from two adjacent sections)

In *Vallonia* it has not been possible to trace any definite sequence of events following early impregnation. It seems improbable that it can have any significance in fertilization; rather, the fate of these early penetrating spermatozoa is a rapid absorption. At times indefinite elongated bodies are seen in oöcytes of various sizes which have somewhat the appearance of sperm cells in process of dis-

integration, although other interpretations are possible. They are seldom found in full-grown oöcytes, and it seems likely that any penetration in early stages may be due to the follicular membranes not being well formed, and that when these are fully developed they make an effective barrier against insemination of the egg until after ovulation. I have never discovered any cleavage stages or even maturation spindles in oöcytes while they remained in the ovotestis. It seems clear that the definitive insemination resulting in fertilization does not occur until the female cells have become detached from the acinus walls.

*The 1.3-mm. Stage* (Pl. III, Fig. 4)

The principal changes noted in the 1.3-mm. stage are a widening and upward extension of the gland and the development of a more lobate form. There is also an increase in size of the oldest oöcytes as compared with those of earlier stages, and with the widening of the lumen the characteristic grouping of male cells in "cell nests" becomes apparent.

*The 1.5-mm. Stage* (Pl. III, Fig. 5)

In the 1.5-mm. stage a division into primary acini is indicated, accompanied by a further increase in size of the oöcytes and of the gland as a whole.

*The 1.8-mm. Stage* (Pl. III, Fig. 6)

In animals measuring 1.8 mm. there occur further enlargement and a subdivision of the gland into lobules, and the oöcytes approach the maximum size of those in the adult gland. New generations of smaller oöcytes continue to appear in the active medullary region. The lumen is almost completely filled with groups of developing male cells representing practically all stages of maturation.

*The 2.0-mm. Stage* (Pl. III, Fig. 7; Pl. IV, Fig. 4; text Fig. 1)

Two millimeters is the average adult size, and when the young animal reaches this diameter the ovotestis corresponds in structure to that already described for the active mature individual. Egg laying follows very shortly, as was observed in my previous study (Whitney, 1938), so that sections may show not only large oöcytes filling up the acini of the hermaphrodite gland but a fertilized egg in the outer genital passage, invested with albumen and shell and ready to be laid (Pl. I, Fig. 2).

MATURATION OF THE GERM CELLS

The maturation of the germ cells of Pulmonata and of other hermaphrodite mollusks has been the subject of a great amount of careful investigation. Since the present study does not deal with detailed observations of chromosome structure, or of the nebenkern, mitochondria, or other cytoplasmic inclusions, no attempt will be made to review this extensive literature in any detail. Most authors agree on the origin of male, female, and nutritive cells from an indifferent germinal epithelium. Both internal genic factors and external influences, such as proximity to nutritive sources, have been cited as sex-determining (Ancel, 1902; Buresch, 1911; Gatenby, 1917, 1918, 1919a and b; Pennypacker, 1930).

Demoll (1912), Alexenko (1928), and Perrot (1930) have described heterochromosomes and have followed their course through spermatogenesis and oögenesis. The manner in which these operate to determine sex remains, however, somewhat hypothetical. A considerable section of the literature has to do with the production of atypical spermatozoa and abortive ova. This literature has been reviewed by Furrow (1935, 1937), who summarizes the chief theories concerning the significance of these abnormal forms. In some studies atypical spermatozoa have been found to effect a partial fertilization (Hyman, 1925; Portmann, 1930). By penetration or contact they seem to determine which eggs are destined to have a merely nutritive function. The usual fate of abnormal spermatozoa, however, is degeneration, and, on the whole, Furrow is inclined to regard them as the result of the interaction of two widely differing germinal tissues in the same gland. They represent a sort of rudimentary hermaphroditism which might be expected in forms intermediate between hermaphroditism and definite dioecism. This is found even in higher animals such as the frog, which in some instances shows a tendency to develop ovarian tissues in the testes. According to Ankel (1930), this labile sex condition would be expected to result in dimorphism of eggs as well as sperm, a dimorphism manifested by an inability of a certain number of eggs to develop. He points out that degenerating eggs are very common in prosobranch ovaries and sometimes serve as nutritive material.

In *Vallonia*, though there is some variety in the appearance of the cells and though unusual-looking spermatozoa have now and

then been found, no regular dimorphic series have been identified. If such dimorphism exists, the atypical forms must be quite rare. The majority of cells observed can be readily classified as belonging to the normal spermatogenetic series. Degenerating ova are common, as previously mentioned.

### *Spermatogenesis*

The spermatogenesis of *Vallonia pulchella* is apparently typical. The stages are figured (text Figs. 4a–r), so that only their general features will be discussed.

*Spermatogonia.* — The first male cells identified in the interior of the young gland, while it is still in the form of a solid rudiment, are interpreted as being primary spermatogonia (text Figs. 4a–c). They are less easily found and identified in later stages, since they tend to occur singly and are not numerically prominent among the maturation stages. As compared with the indifferent cells of the earlier period, they are large, having a long diameter of 8–10 microns. They are frequently pedicellate, with the base directed toward the germinal epithelium or follicular membrane, and the wider portion of the cell, which contains the nucleus, facing the lumen. A number of them show mitotic figures, but chromosomes are difficult to count, owing to their small size and crowded condition. The counts made ranged from 36 to 38.

Smaller spermatogonia, 5–6 microns in diameter, are identified as secondary, being derived from the primary stages by mitotic divisions (Figs. 4d–e). Their chromatin is less finely dispersed, and they have a more rounded and compact appearance. They occur in groups suggesting recent divisions, whereas the primary spermatogonia, as stated above, are more likely to be found singly.

Primary spermatogonia are interpreted as originating from germinal epithelial cells, by a process of growth and differentiation. They are the first male cells shed into the lumen, where they give rise to secondary spermatogonia by repeated mitoses.

*Spermatocytes.* — Primary spermatocytes are numerous in mature glands during the active season. They are large cells 10–12 microns in diameter, and, like the spermatogonia, may be pedicellate. In section the cytoplasm forms a narrow border around the large nucleus. Typical preleptotene, leptotene, and bouquet stages are found (Figs. 4f–h). The tetrad stages (Fig. 4i) offer the best oppor-

FIG. 4. Spermatogenesis: *a*, primary spermatogonium; *b*, primary spermatogonium in prophase; *c*, chromosome plate of primary spermatogonium, semiprofile view; *d*, group of secondary spermatogonia; *e*, chromosome plates of secondary spermatogonia; *f*, primary spermatocyte-preleptotene stage; *g*, leptotene stage; *h*, bouquet stage; *i*, tetrad stage; *j*, first maturation division, profile view; *k*, chromosome plate of secondary spermatocyte; *l*, second maturation division, profile view of metaphase; *m*, anaphase; *n*, telophase; *o*, spermatids; *p*, spermatids beginning metamorphosis; *q*, late stage of spermateliosis; *r*, group of mature sperm heads. (Selected from sections shown in Plate IV and text Figs. 1 and 3 and from other sections)

tunity for chromosome counts, since the chromosomes are better spaced in the large nucleus than in any other stage. On the average, about 20 tetrads have been counted.

Maturation divisions (Figs. 4j–n) evidently occur in rapid succession without the intervention of a resting stage, to judge from the infrequency with which these stages are encountered. These findings correspond with those of Demoll (1912) for *Helix*. Profiles of the two divisions are rather similar except for the differing sizes of the cells (Figs. 4j, l). The secondary spermatocytes measure 8–9 microns and show the small rounded chromosomes somewhat closely grouped in a dense homogeneous cytoplasm, without a nuclear membrane (Fig. 4k). A few chromosome counts were obtained. They ranged from 18 to 20.

*Spermatids.* — The spermatids (Figs. 4o–p) are small cells, 6–7 microns in diameter, and somewhat angular in outline as compared with cells of previous stages, probably owing to their being closely massed together in rather large groups often consisting of several dozen cells. They evidently remain in this stage for a considerable time, and are readily identified because of their uniform and characteristic appearance. They constitute a reserve from which a few mature spermatozoa are formed by metamorphosis from time to time.

*Spermateliosis* (Figs. 4p–r). — The cells are so small that little can be said of the details of metamorphosis. The spermatid elongates; the nucleus assumes a tapering form and comes to occupy one end. In some preparations an axial fiber is faintly visible. In later stages a spiral twisting of the sperm head occurs, a development described for other forms also (Byrnes, 1900; Woldt, 1932), and a spindle-shaped mass of cytoplasm is discarded from the tail. These discarded fragments apparently disintegrate, and may contribute to the nourishment of the maturing cells, as mentioned above.

*Spermatozoa* (Fig. 4r). — The mature spermatozoa, including the tail, may have a length of 60–70 microns. They usually occur in relatively small groups of about a dozen cells, but there are isolated ones also in various parts of the gland lumen. The tail is infrequently seen. It is probably lost before the sperm cell becomes functional, as authors have reported for other mollusks. We have already noted that spermatozoa may be present in the hermaphrodite glands of juvenile animals in which oöcytes have reached only a fraction of the maximum size.

### *Oögenesis*

*Origin of the female cells.* — Ancel (1902) claims a unicellular origin for the egg cell of *Helix*. He expresses the view that each

female cell is formed directly by a process of growth and differentiation from a germinal epithelial cell, without a preliminary multiplication through oögonial divisions. To quote his account, "As soon as the female cell is recognizable . . . it is in the growth stage, — one cannot differentiate in the young gland of *Helix* a female cell that is not an oöcyte. The oöcyte arises directly from an epithelial cell to which one cannot give at any time the name primordial ovule or ovogonium." Essentially the same opinion is recorded by Buresch (1911), Gatenby (1917), and Pennypacker (1930). Although Pennypacker found certain cells in *Polygyra* which she was inclined to interpret as being oögonia, she could not identify them with certainty. Buresch points out that in general the function of the oögonial divisions is to increase the number of eggs and thereby the chances of survival, but in *Helix* he notes other protective features which make the production of a large number unnecessary: the eggs are enclosed in a hard shell; they are deposited deep in the earth; and the young emerge well developed, protected by the body shell.

In some mollusks oögonial divisions have been reported, but in *Vallonia* the situation evidently agrees with that in *Helix* and *Polygyra*, and the female cells are first recognized as oöcytes. No definite cells can be distinguished as true oögonia. Few eggs are produced, and various protective features associated with egg laying and development may be postulated as making a large number unnecessary, much as in the case of *Helix*.

*Growth period.* — The first recognizable oöcytes are found in the periphery of the young gonad while it is still in the early compact stage (Pl. IV, Fig. 2). They stand out from the spermatogonia because of the larger proportionate size of the cytosome to the nucleus, their elongated form suggestive of ameboid migration, and their prominent double nucleoli, the so-called amphinucleoli. One of these enlarges greatly during the growth period, the other much less. The larger of the nucleoli is regarded as being plasmatic; the smaller one, a true nuclear nucleolus.

The nucleus stains more lightly than the surrounding cytoplasm, and as growth progresses the nuclear chromatin, irregularly dispersed, tends to lose its staining properties to some degree and shows up as rather faint fluffy masses, similar to the condition described for *Polygyra* by Pennypacker (1930).

Full-grown oöcytes, as seen in their later position in the acinus

walls, have irregular polygonal forms because of the pressure of adjacent cells. They measure 70–75 microns in their longest diameter. The nucleus tends to have a rounded outline, but in some of the largest cells it may be quite irregular. This may indicate an approach to the breaking up in preparation for the maturation divisions. But I have never found any maturation spindles or cleavage stages while the female cells remained in the ovotestis of *Vallonia*, and a number of authors similarly record the absence of these (Byrnes, 1900; Larambergue, 1930; Pennypacker, 1930; Woldt, 1932). An exceptional case is that recorded by Lams (1910), who describes an intraovarian insemination in *Arion empiricorum*. He observed not only maturation divisions but segmenting eggs, morulae, and blastulae, in the interior of the gland.

*Maturation divisions.* — It seems evident that in *Vallonia*, as in the majority of the forms cited above, maturation divisions do not take place until after ovulation. The breaking up of the egg nucleus and the formation of the maturation spindle must be accompanied by a very rapid passage of the egg from the hermaphrodite gland into the outer genital passage. A number of authors record their failure to find ova in the hermaphrodite duct. In the present study maturation divisions have been observed only in eggs located in the outer passage, invested with albumen and shell, and ready to be laid. Such an egg is figured (text Fig. 5a) with what is interpreted as a polar view of the first maturation spindle, showing large asters and 32 chromosomes. The diploid number indicated by other counts is nearer 40. In this cell it may be that only part of the chromosomes have separated or that some are irregularly crowded together and difficult to distinguish.

Figure 5b shows an egg similarly situated in the uterus, with the second maturation division almost completed. The first polar body has migrated a short distance into the albumen. The second polar body still adheres to the surface of the egg, being held by remnants of spindle fibers. The egg chromosomes form a straggling group, not yet reorganized into the female pronucleus, and the sperm head, still small and compact in a little clear area near the periphery of the egg, has not yet begun its development into a male pronucleus. Indications are that the polar bodies have been given off in rapid succession, since the chromosomes of the first polar body are still clear and distinct. Counts of 17 and 18 have been made in several

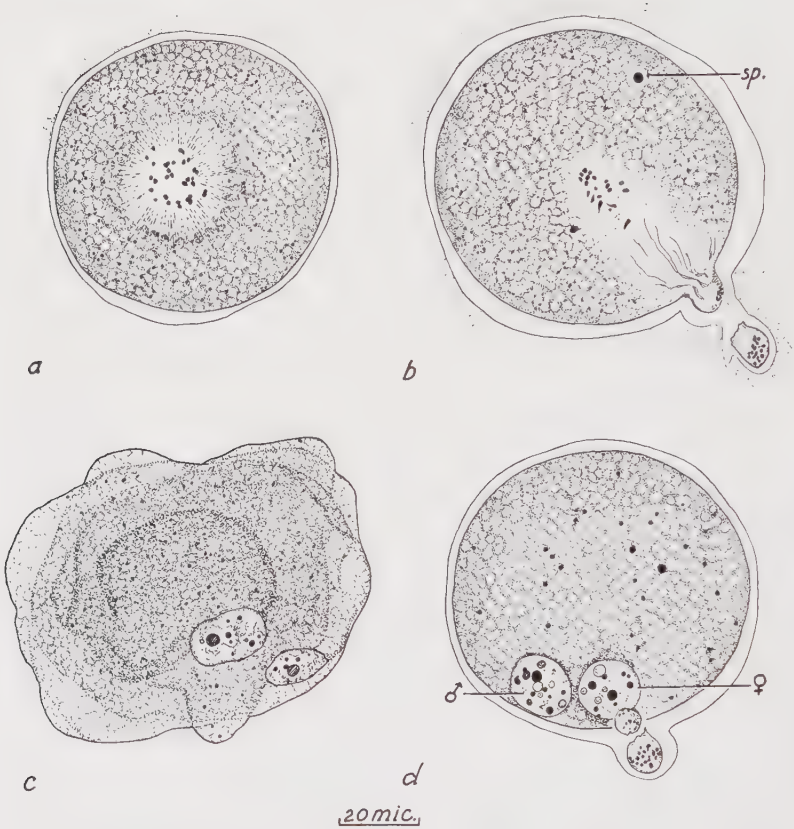


FIG. 5. Fertilized eggs found in outer genital passage: *a*, first maturation spindle, polar view of metaphasic chromosome plate; *b*, second maturation division — two polar bodies, egg chromosomes, and head of spermatozoon (*sp.*) shown — both *a* and *b* surrounded with albumen layer and shell; *c*, *d*, two fertilized eggs found in the same individual — *c* in the region of the albumen gland but lacking the albumen layer, *d* with albumen and shell — both showing male and female pronuclei. (All reconstructed from two or more adjacent sections)

instances. The chromosomes of the second polar body are smaller and less distinct. Few examples were obtained which were favorable for the counting of egg chromosomes. Those in Figure 5b number about 20.

### INSEMINATION

The intraovarian insemination noted in *Arion empiricorum* by Lams (1910) is exceptional among gastropods. In most studies authors have found insemination delayed until after oöcytes have left the acini. In *Vallonia* I have not observed actual penetration of spermatozoa, except possibly into early oöcytes (Pl. IV, Fig. 3), but it seems most reasonable to suppose that the definitive insemination occurs as the ova leave the acinus wall and traverse the medullary portion of the ovotestis on their way into the hermaphrodite duct. It is here, in the lumen of this medullary region, that male cells in all stages, including ripe spermatozoa, are abundant during the active season, and even a very rapid passage through this part of the gland would afford ample opportunity for insemination. Monospermy is evidently the rule. I have found no supernumerary spermatozoa in the uterine eggs or in the albumen surrounding them.

### FERTILIZATION

Good descriptions of fertilization processes in pulmonates have been contributed by Mark (1881) for *Limax campestris,* Garnault (1888–89) for *Helix aspersa* and *Arion empiricorum,* Byrnes (1900) and Linville (1900) for *Limax* species, Lams (1910) for *Arion empiricorum,* Crabb (1927) for *Lymnaea stagnalis appressa,* Larambergue (1930) for *Bullinus contortus,* and Perrot (1937) for *Helix pomatia.* There is a general similarity in these accounts. In the study of *Vallonia* it has not been possible to assemble a complete series of stages showing progressive steps in the development of the two pronuclei. The fact that eggs are deposited singly and at varying time intervals makes the obtaining of favorable stages a rather fortuitous matter. In only one instance have two eggs been found outside the hermaphrodite gland in the same individual. The first, enclosed in albumen and shell, occupies the lower passage (Fig. 5d). The second is farther back, in the region of the albumen gland but still lacking the albumen layer, and has an irregular form (Fig. 5c), which recalls a suggestion of Ikeda (1937) that ova make their way out of the hermaphrodite gland by means of ameboid movements. It has well-developed male and female pronuclei, like its predecessor, so that there is apparently no strict correlation between the progress

of maturation and fertilization and the level at which the ovum is found.

Such stages as have been obtained indicate that fertilization processes in *Vallonia* follow in a quite regular manner those of other pulmonates referred to above. There is only one spermatozoon in fertilized eggs; it appears first as a small, compact sperm head (Fig. 5b), but later transforms into a male pronucleus (Figs. 5c–d). The majority of the fertilized eggs in the uterine passage present in section an appearance like that of Figure 5d. They are invested with a thick albumen layer and shell, and are spherical, possibly because of suspension in the liquid medium, and also in part because of cortical changes accompanying fertilization. They measure 70–75 microns in diameter, which is the approximate size of the largest oöcytes while still in the hermaphrodite gland. This indicates that no growth takes place between the gland and the uterus. The cytoplasm is vesicular in appearance and contains small spherical yolk granules which stain black with iron hematoxylin.

The male and female pronuclei are typically in close juxtaposition at the animal pole. The male pronucleus in some preparations is slightly larger than the female and slightly elongated. The female pronucleus may be identified by the proximity of the two polar bodies. In both pronuclei the chromatin is in large, rounded, nucleoli-like masses, with smaller granules interspersed. This appearance must be quite characteristic, since it is noted repeatedly in the studies mentioned above.

In a few individuals cleavage has been found to occur while the egg is still in the oviduct. This may indicate an incipient tendency toward ovoviviparity. The eggs are retained in oviducts for varying periods, so that successive cleavages may take place. One egg fixed with the parent animal just as it was being laid shows indications of gastrulation. Others fixed soon after deposition are still in morula and blastula stages.

The evidence of typical chromosome reduction, the regular appearance of two polar bodies as well as two pronuclei, and the similarity of the stages studied to normal stages described for other pulmonates, together with the absence of the vas deferens and failure to observe copulation, all indicate that self-fertilization is the normal and usual process of reproduction in *Vallonia*.

DISCUSSION

One is impressed with the similarity of conditions in *Vallonia* to those of other Pulmonata, both in general and as regards many details. Specializations are noted, however, some of which at least seem to be due to restrictions of small size and others to habit and habitat. The large size of the eggs in proportion to that of the animal, their reduction in number, and protective features insuring juvenile survival are very striking. The eggs must be laid singly because of the small size of the animal, and egg laying is dependent on temperature and moisture. All land animals have to cope with problems of desiccation and extremes of temperature, and this is particularly true of a surface-living form such as *Vallonia*, which does not burrow to any extent, as does *Helix*, for example. These limiting factors are offset by the protracted breeding season (eggs have been collected out of doors from May to October) and by the readiness with which they are deposited whenever favorable conditions present themselves. Such conditions evidently induce a general activity of the germ gland, with the result that male and female cells in various stages of maturation are to be found in it at practically all times, although in an arrested condition during cold and dry periods. It is thus possible to bring some of them to maturity very quickly, as witness the readiness with which eggs are deposited in the laboratory by specimens brought in during the winter. Reproduction is evidently not complicated by alternating male and female phases or any such cyclical condition. Periods of drought and low temperature are to be regarded as enforced seasons of reproductive cessation rather than any functional break in the process.

The absence of male ducts, making self-fertilization obligatory, represents a further simplification both structurally and functionally and, as has been suggested in other studies, may have favored survival and propagation. I have found populations of *Vallonia* year after year in the same restricted area and at times very congested. This I attribute to the limited migratory powers of the animal and to artificially favorable moisture conditions around dwellings which make possible more continuous egg laying and a consequent piling up of populations.

Human agencies also multiply opportunities for accidental dispersal which would be advantageous to a slow-moving form, and

the power of self-fertilization would enable any isolated individual transported into a new region by such means to start a new colony.

SUMMARY

This paper presents histological studies of the hermaphrodite gland of *Vallonia pulchella* Müll. as correlated with the life cycle and habits of the animal. The following features are brought out:

1. The ovotestis of the mature animal has the form of an acinous or digitate structure, with a number of cysts opening into a common lumen and joining the hermaphrodite duct, as is typical of many hermaphrodite mollusks. The efferent ducts have not been studied in detail, but the absence of the vas deferens was noted in sections, in agreement with the findings of previous authors. The absence of copulation is thus explained, and also the fact that fertile eggs are laid as freely by isolated individuals as by those in mass cultures.

2. The microscopic features of the gland differ according to the seasonal activity. Material fixed during the active summer season shows all stages of maturation; dormant winter specimens indicate an arrested condition of the gland, whereas spring and fall material exhibits intermediate conditions. Both male and female cells, however, are present during all seasons. There is no evidence of an alternation of male and female phases or of any cyclical condition other than the enforced cessation of reproductive activity during cold and dry periods.

3. The ovotestis develops from a compact cellular body, which was first distinguished at about the time of hatching and which is composed of indifferent germ cells. It later assumes the form of a single hollow vesicle, which subsequently takes on a lobate structure and becomes subdivided into the acini composing the adult gonad. Accompanying this development is a differentiation of male, female, and nutritive (or follicle) cells from the indifferent cells of the early gonad. They continue to arise from time to time in adult life, whenever conditions are favorable.

4. Male cells, which are first identified as primary spermatogonia, undergo their maturation and development in the lumen of the ovotestis. Female cells, first identified as oöcytes, develop in the walls of the acini, held in place by follicular membranes produced by follicle and nurse cells. The medullary portion of the gland is

the most active in the production of new elements. There is apparently a migration of small oöcytes formed in this region into the acini, where the remainder of their growth period is spent.

5. There are mature spermatozoa in the gland at an early stage, when the animal is about half grown; full-grown oöcytes do not occur until adult size is reached, at which time egg laying begins. There is evidence of some penetration of young oöcytes by spermatozoa, but such spermatozoa apparently disintegrate and do not result in fertilization.

6. Maturation divisions and effective insemination of the eggs do not occur until after they leave the acinus wall.

7. Usually only one fertilized egg is found in the uterus at any one time, since the small size of *Vallonia* prevents the laying of more. Monospermy occurs, which leads to the formation of a typical male pronucleus.

8. Two polar bodies are regularly given off, either shortly before or after the egg becomes invested with albumen and a calcareous shell, and a female pronucleus develops along with the male pronucleus in a typical manner. Cleavage may begin before the egg is laid.

9. Chromosomal counts made in spermatogonia, primary spermatocytes, secondary spermatocytes, first polocytes, and eggs give a haploid number of 18 to 20.

10. Histological evidence from this study, including the presence of mature sperm and eggs in the same acini at practically all times, the absence of the vas deferens, typical synapsis and chromosome reduction, the formation of two polar bodies and the development of two pronuclei, supports previous studies in establishing self-fertilization as the normal method of reproduction in *Vallonia pulchella*.

11. In general, reproductive conditions are effectively adjusted to the small size of the animal and to environmental factors, and are apparently favorable to its distribution and survival.

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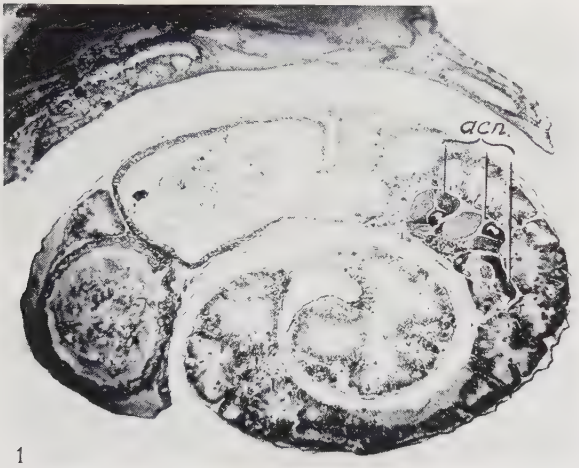
## EXPLANATION OF PLATE I

Abbreviations: *acn.*, acini; *al.*, albumen of egg; *al. gl.*, albumen gland; *fert. egg*, fertilized egg; *ut.*, uterus

Fig. 1. Sagittal section through mature *Vallonia pulchella*, showing the position of the hermaphrodite gland and the three groups of acini composing it

Fig. 2. Sagittal section of mature *Vallonia pulchella*, showing a fertilized egg in the uterus, ready to be laid

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## EXPLANATION OF PLATE II

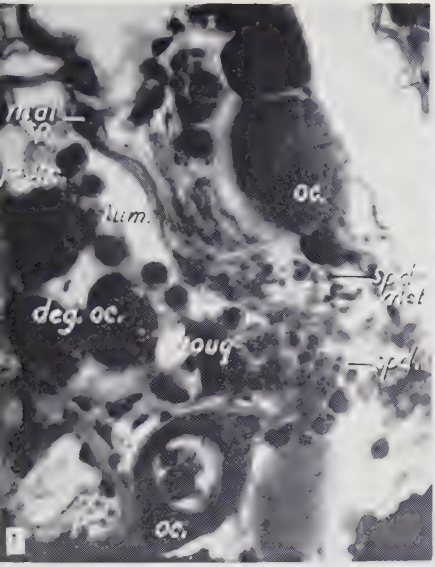
Conditions of the hermaphrodite gland at different seasons of the year

Abbreviations: *bouq.*, bouquet stage; *deg. oc.*, degenerating oöcytes; *f.*, follicle; *f. c.*, follicle cells; *f. m.*, follicular membrane; *lum.*, lumen; *mat. sp.*, mature spermatozoa; *oc.*, oöcytes; *pr. spc.*, primary spermatocytes; *spd.*, spermatids; *spd. met.*, spermatids in metamorphosis

Fig. 1. Active medullary portion of gland in individual collected in July

Fig. 2. Portion of gland in dormant individual collected during January

Figs. 3–4. Portions of the glands in individuals collected in April and October respectively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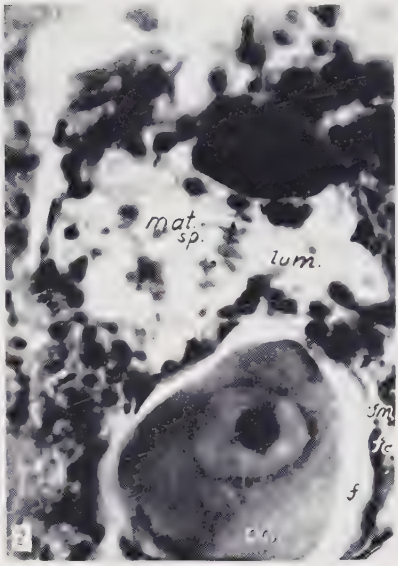
deg. oc.
oc.
1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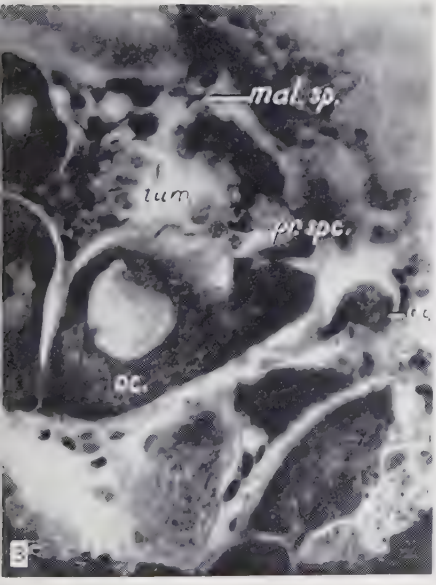
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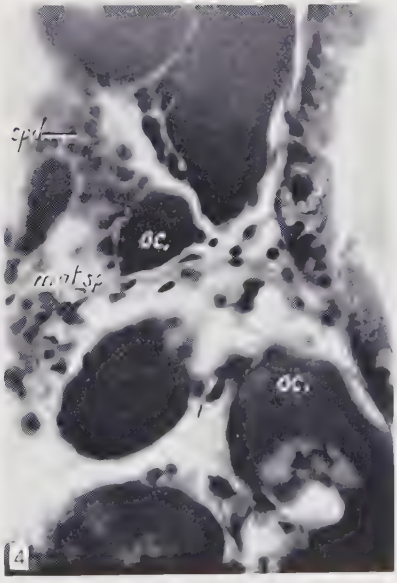
spd.
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mat. sp.
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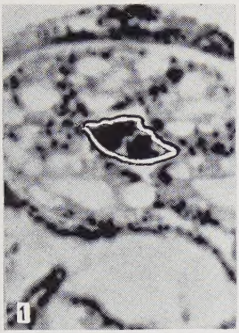
## EXPLANATION OF PLATE III

Series showing development of the hermaphrodite gland from the time of hatching up to and including adult size. Stages: Fig. 1, 0.6 mm.; Fig. 2, 0.9 mm.; Fig. 3, 1.0 mm.; Fig. 4, 1.3 mm.; Fig. 5, 1.5 mm.; Fig. 6, 1.8 mm.; Fig. 7, 2.0 mm. Measurements represent the widest shell diameter of the alcohol specimens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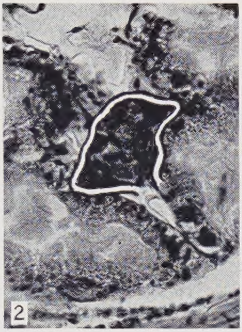
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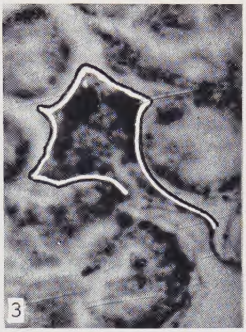
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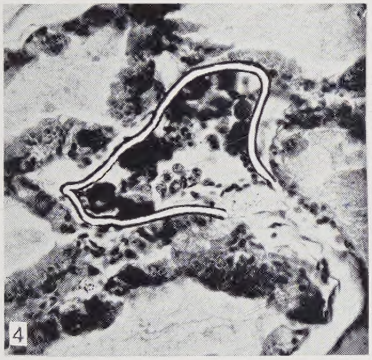
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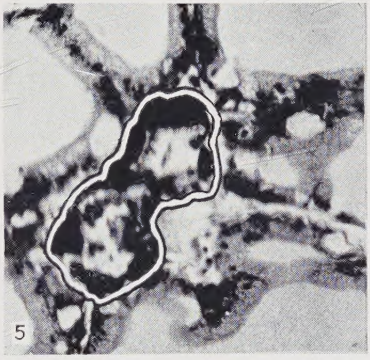
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## EXPLANATION OF PLATE IV

Oil-immersion studies of developmental stages

Abbreviations: *deg. oc.*, degenerating oöcytes; *f.*, follicle; *f. m.*, follicular membrane; *h. d.*, hermaphrodite duct; *lum.*, lumen; *mat. sp.*, mature spermatozoa; *oc.*, oöcytes; *prim. g. c.*, primordial germ cells; *pr. spc.*, primary spermatocytes; *pr. spg.*, primary spermatogonia; *sec. spc.*, secondary spermatocytes; *sec. spg.*, secondary spermatogonia; *spd.*, spermatids

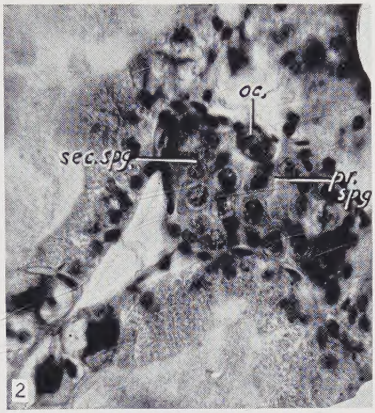
FIG. 1. Undifferentiated early gonad shortly after hatching

FIG. 2. Beginning of differentiation, as seen in the 0.9-mm. individual (early oöcytes at the periphery; early maturation stages of male cells in the interior)

FIG. 3. Further differentiation as seen in a half-grown (1.0-mm.) snail; ripe sperm present, some penetrating into the young oöcytes

FIG. 4. Portion of mature gonad, showing full-grown and smaller oöcytes and male cells in various stages of maturation

prim. g.c.
1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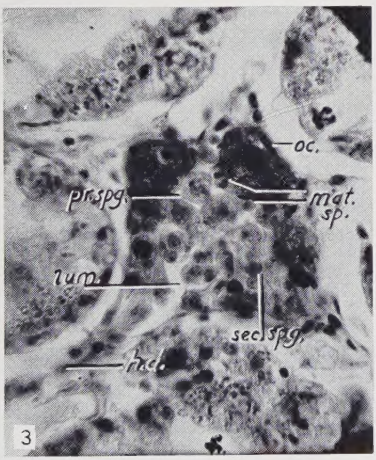
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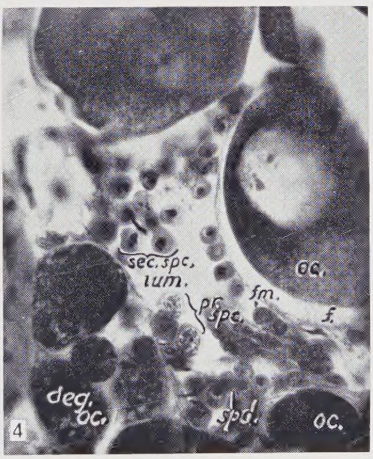
3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4